Prolactin in the Postpartum Rat: Synthesis and Release in the Absence of Suckling Stimulation

Abstract. *Postpartum female rats deprived of suckling stimulation while being kept continuously with pups nonetheless suspended ovarian cyclicity for an average of 16 days and responded to uterine trauma by forming deciduomata. These results show that the extramammary stimulation afforded by young is alone capable of sustaining prolactin output from the adenohypophysis of the postpartum rat.*

Postpartum female rats deprived of suckling stimulation by thelectomy (surgical removal of nipples) continue to crouch over their young in a posture closely resembling actual nursing (*1*). Nursing (or the nipple excitation accompanying nursing) sustains the discharge of prolactin (*2*). Sight, sound, odor, and perhaps "feel" of the young may alone be sufficient to sustain this same discharge, as was suggested by Grosvenor's (*3*) report that lactating female rats separated from their young by the interposition of a wire-mesh screen responded, after 30 minutes, with a significant depletion of pituitary prolactin stores.

Grosvenor's study, however, does not provide information concerning the role of pup-related stimuli in sustaining prolactin output; it offers data relevant only to the short-term release of the hormone; nor are the effects of pup-related stimuli distinguished from those of suckling. Grosvenor's subjects had been actively suckled for some 14 days before being tested. Thus, the sight, sound, and perhaps odor of the young may have become associated with the discharge of prolactin induced by suckling.

We monitored the luteal activity of female rats deprived of suckling stimulation while in the presence of young. Female rats of the Wistar strain, born and reared in our laboratory, had been maintained from the day of birth under a reversed day-night cycle of 12 hours of light and 12 hours of darkness, and under a relatively constant temperature of 20°C. At the time of the observations and measurements the animals were primiparous and between 110 and 150 days of age.

The nipples of ten female rats, 21 to 30 days of age, were excised. In a group of comparable age, we performed a "sham thelectomy": two 4-mm incisions in the ventral skin, in a line as far as possible from any of the nipples. Both groups, in addition to a group of 15 unoperated females, were subsequently impregnated and allowed to give birth normally.

Within 12 hours after they had given birth, unoperated females were deprived of all young. Thelectomized and sham-thelectomized females, likewise deprived of their own young, had continuously available thereafter a litter of six foster pups. These litters were supplied by a battery of "donor females" impregnated at the same time as the thelectomized and sham-thelectomized females.

To insure the health and vigor of the young given to the thelectomized females, their litters and, for purposes of control, the litters of the sham-thelectomized females were returned to their natural mothers for at least 12 hours per day, during which time other litters from other donor females became available for transfer.

Because prolactin is essential in supporting luteal activity in the rat and hence in suspending gonadal rhythmicity, the duration of the postpartum diestrus serves as index of prolactin output. Accordingly, we took daily vaginal smears from each female until a predominantly cornified cellular picture was obtained. Such a picture defined the 1st day of estrus and the resumption of ovarian cycling. (The immediate postpartum estrus was ignored.)

Unoperated animals whose pups had been removed began to recycle after an average of 7 days. Thelectomized and sham-thelectomized females, all of whom had been continuously in the presence of pups, suspended gonadal cyclicity for a significantly longer time —16 and 20 days, respectively. (In each comparison, the Mann-Whitney *U*-value was less than .05.) These data leave no doubt that pup-related stimuli, without any previous association with suckling, can function to extend the diestrus of the postpartum rat (thelectomized compared to unoperated—with pups removed). Furthermore, suckling (or the full stimulative complex of which suckling is an integral part) has an effect of even longer duration (sham-thelectomized as compared to thelectomized).

Table 1. Median cornual weights for each group after uterine trauma.

Female rats	Weights (mg)		$\frac{T-C}{C}$
	Traumatized horn (T)	Control horn (C)	
Thelectomized	753.4	181.7	3.26
Suckled	701.5	152.1	2.75
Removed	429.3	279.5	.07

However, as exteroceptive stimuli, sight, sound, odor, and perhaps "feel" of the young, although able to promote the output of prolactin in amounts sufficient to suspend cyclicity for some 16 days, may not sustain titers sufficient to support full luteal activity, as suckling normally does. Did the thelectomized females, when cyclicity was arrested, differ from unoperated lactating females in regard to luteal functioning? The decidual cell reaction after uterine traumatization was used for endocrinological comparison as follows.

Another group whose nipples had been excised was also subjected to a daily regimen of pup transfer ($N=12$). However, instead of being tested for the resumption of cycling, these females underwent uterine trauma on day 5 postpartum. Traumatization consisted of scratching the antimesometrial luminal surface of one uterine cornu while the animal was under light ether anesthesia. Within 2 hours after the operation, these females were returned to their litters. After 5 days they were killed and both cornua were removed and weighed individually to the nearest 0.1 mg.

The same procedure was used on two other groups (14 and 15 animals). The first group consisted of unoperated lactating females kept continuously in the presence of young; the second consisted of lactating females deprived of all young within 12 hours after having given birth.

Table 1 shows the median cornual weights obtained from thelectomized and suckled females as well as from females who had all pups removed within 12 hours after parturition. The Mann-Whitney *U*-test revealed that thelectomized and suckled animals did not differ from each other in response to uterine trauma, whereas both groups differed significantly from unoperated females deprived of young ($P < .01$).

Thus, the titer of prolactin synthesized and released in response to pup-related stimuli can keep gonadal rhythmicity in abeyance for some 16 days, and can sustain luteal activity capable of supporting full decidualization. Two correlative questions remain to be investigated: How do pup-related stimuli, without previous association with the discharge of prolactin induced by suckling, acquire certain properties; and through which neurohumoral pathways do these stimuli operate to influence gonadotrophic activity?

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3. C. E. Grosvenor, *Endocrinology* **76**, 340 (1965). This same possibility was suggested some 35 years ago by B. P. Wiesner and N. M. Sheard [*Maternal Behaviour in the Rat* (Oliver and Boyd, London, 1933)].

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Hypothalamic Motivational Systems: Fixed or Plastic Neural Circuits?

We have described (*1*) a procedure for modifying the behavior elicited by hypothalamic stimulation. The new behavior competed effectively with the initial behavior and was elicited by the identical stimulus parameters, and we concluded that "there is considerably more plasticity in establishing connections between hypothalamic circuits and motivated behavior than commonly advanced interpretations of 'stimulus-bound' behavior suggest."

An alternative hypothesis was advanced by Wise (*2*) who reported that, when the current was high enough, electrodes could elicit more than one behavior pattern. Finding that the threshold for eliciting a particular behavior tended to decline over time, Wise concluded that the second or third behavior emerging in our studies with one stimulus intensity resulted from the gradual decline in threshold of the neural circuits responsible for the behavior. Wise maintains that there are separate "fixed neural circuits, functionally isolated from each other," and that the threshold changes in these circuits create the impression of plasticity. Since this argument may appeal to those who think that the hypothalamus contains discrete neural circuits related to each motivational system, we feel impelled to reply. Our procedure was misunderstood, and additional supporting experimental data (not available to Wise) prevent us from accepting the alternative explanation of our results.

Wise assumed that the first behavior pattern which we observed in response to stimulation was elicited at threshold currents that were obtained by gradually raising the intensity. The implication is that if we used suprathreshold currents, the second behavior would have been observed from the beginning of the experiment. Our procedure did not involve threshold values, and we did not state that it did. Indeed, others have thought that we had obtained several behavior patterns from stimulation at the same site because the current was too high. In our first report, the current was two to three and one-half times the threshold for eliciting the behavioral response. Only subsequently have we used threshold values (we obtained similar results). Thus, our earlier results were criticized both because the current used was too high and because the current used was too low.

In other experiments (not included in our abbreviated report in *Science*), we either raised the current as high as possible without damage to the animal, or stimulated the animal over several weeks of testing at the first current level (*3*). In most cases, when the first goal object to which the animal responded was still available, a second stimulus-bound behavior pattern was not displayed. In animals stimulated over several weeks, the threshold changes reported by Wise should have occurred.

Only after we removed the first goal object did the second behavior pattern gradually emerge. In the experiment reported by Wise, the first goal object was removed when the current was raised. We used stimulation without the initially preferred goal object (and did not manipulate the current) to obtain a second behavior pattern. Wise's procedure confounds current manipulation with the effect of removing the initially preferred goal object. No quantitative or qualitative information is provided on the time course of emergence of this second behavior. Nor does Wise consider the difficulties posed for the position of completely independent neural circuits by the fact that a second behavior pattern is hard to demonstrate even at high current levels, in the presence of the first goal object.

In our experience the emergence of the second behavior, even at higher current levels, may take several hours of intermittent stimulation. Once this new behavior is associated with the stimulus, it is possible to elicit it with lower current. The relevant point is that it is not the stimulation which produced the lower threshold, but the acquisition of the behavior pattern.

The threshold for eliciting behavior by electrical stimulation may decline over successive test sessions. Many factors probably contribute to this decline; among these are variables related to an increased readiness to respond in a particular way and factors related to stimulus-generalization gradients. We noted this decline in behavioral threshold in the context of self-stimulation experiments, and cautioned against assuming that the excitability of the neural elements directly activated by the electrical stimulus are responsible (*4*). Wise seems to imply that the repeated stimulation lowers the threshold, and has overestimated the amount of stimulation necessary to produce a second behavior in our experiment. We had written that most animals required only one night of intermittent stimulation for the new behavior to emerge, and in subsequent experiments a much shorter period was often required. Wise implied that our experiments usually involved several nights of stimulation. Furthermore, our statement, "The earlier the onset of the first behavior during the preliminary stimulation sessions and the more consistently this behavior was displayed, the sooner the animal switched to a second behavior pattern . . ." has been ignored, apparently because it is not consistent with the hypothesis that the stimulation per se is responsible for lowering the threshold.

Although Wise presented some useful data, we still maintain that the relationship between the activation of hypothalamic neural circuits and stimulus-bound behavior is plastic. This conclusion has been strengthened by further experimentation with a greater variety of behavior patterns (*3*).

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